

EXHIBIT 8

The *MDR1* (ABCB1) Gene Polymorphism and its Clinical Implications

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Abstract

There has been an increasing appreciation of the role of drug transporters in the pharmacokinetic and pharmacodynamic profiles of certain drugs. Among various drug transporters, P-glycoprotein, the *MDR1* gene product, is one of the best studied and characterised. P-glycoprotein is expressed in normal human tissues such as liver, kidney, intestine and the endothelial cells of the blood-brain barrier. Apical (or luminal) expression of P-glycoprotein in these tissues results in reduced drug absorption from the gastrointestinal tract, enhanced drug elimination into bile and urine, and impeded entry of certain drugs into the central nervous system. The clinical relevance of P-glycoprotein depends on the localisation in human tissues (i.e. vectorial or directional movement), the therapeutic index of the substrate drug and the inherent inter- and intra-individual variability.

With regard to the variability, polymorphisms of the *MDR1* gene have recently been reported to be associated with alterations in disposition kinetics and interaction profiles of clinically useful drugs, including digoxin, fexofenadine, ciclosporin and talinolol. In addition, polymorphism may play a role in patients who do not respond to drug treatment. Moreover, P-glycoprotein is an important prognostic factor in malignant diseases, such as tumours of the gastrointestinal tract.

A growing number of preclinical and clinical studies have demonstrated that polymorphism of the *MDR1* gene may be a factor in the overall outcome of pharmacotherapy for numerous diseases. We believe that further understanding the physiology and biochemistry of P-glycoprotein with respect to its genetic variations will be important to establish individualised pharmacotherapy with various clinically used drugs.

Introduction of the concepts of pharmacogenetics to the clinical setting has had an impact on individualisation of drug treatment, and could therefore contribute significantly to enhanced drug safety and efficacy. Drug-metabolising enzymes are typical cases that have been intensively investigated. For example, the activity of cytochrome P450 (CYP

2D6 is polymorphically distributed in the population and related to the presence of a number of allelic variants with varying degrees of functional significance. Thus, genotyping is expected to provide a new tool for predicting individual drug-metabolising capabilities before treatment begins. Recently, some naturally occurring polymorphisms of the

MDR1 gene have been reported to be correlated with potential clinical effects,^[1-6] with the levels of P-glycoprotein expression in human tissues,^[7-10] or with the bioavailability of orally administered drugs.^[11] Furthermore, there is some evidence that the extent of P-glycoprotein induction and the interaction profile of P-glycoprotein substrates are dependent on *MDR1* polymorphisms.^[9,11,12]

Numerous candidate single nucleotide polymorphisms (SNPs) have been identified and characterised. Notably, the C3435T mutation in exon 26 has been examined in detail. In addition, the G2677T/A mutation is of interest because it is closely linked to C3435T. However, the effects of these SNPs on the expression of P-glycoprotein and the pharmacokinetic and pharmacodynamic outcomes of certain drugs are unclear.^[13]

At a time when the potential importance of P-glycoprotein function is receiving much attention, this review highlights recent studies by others and ourselves on the role of the *MDR1* gene polymorphism in expression in various human tissues, its pharmacokinetic and pharmacodynamic impacts, as well as the inter-racial variability of allelic frequencies. In addition, possible explanations for the variable and conflicting results seen among studies are discussed. The scope of this review is strictly limited to observations from human studies.

1. General Features

1.1 Localisation in Human Tissues and Basic Functions

P-glycoprotein, a transmembrane transporter encoded by the *MDR1* gene, acts as an efflux pump in an adenosine triphosphate (ATP)-dependent fashion. It was first identified in human cancer cells as a protein responsible for resistance against many anti-cancer drugs.^[14-17] Subsequently, this efflux transporter has been found in various normal human tissues. Expression is identified in the small and large intestinal epithelium, adrenal gland, placenta (trophoblasts), kidney (the brush border of the renal

tubule), liver (the canalicular membrane of the hepatocyte), pancreas (pancreatic ductile cell), and capillary endothelial cells of brain and testes.^[18-22] In these tissues, P-glycoprotein is located on the apical or luminal surface of the epithelial cells. P-glycoprotein has also been found in peripheral blood lymphocytes.^[23,24] On the basis of its tissue distribution and findings in knockout mice, P-glycoprotein is speculated to play an important role in the excretion of foreign xenobiotics and endogenous substrates via the canalicular membrane of hepatocytes into the bile, via the brush border membrane of enterocytes into the gut lumen and via the brush border membrane of proximal tubules into the urine.^[25,26] P-glycoprotein in trophoblasts contributes to the function of blocking the transfer of hydrophobic xenobiotics across the human placenta.^[27] In the endothelial cells of the blood-brain barrier, P-glycoprotein prevents the entry of substrates into the central nervous system.^[28-30] In summary, P-glycoprotein functions as a defence mechanism against xenotoxins.

1.2 Substrate Drugs

P-glycoprotein recognises and transports a variety of drugs, including chemotherapy agents (paclitaxel^[31] and irinotecan^[32]), antibacterials (erythromycin^[33] and levofloxacin^[34]), immunosuppressants (cyclosporin and tacrolimus),^[35] cardiac drugs (digoxin^[36] and quinidine^[37]), calcium channel antagonists (diltiazem^[38] and verapamil^[39]) and HIV protease inhibitors (ritonavir^[40] and saquinavir^[25]). P-glycoprotein substrates, inducers and inhibitors are listed in detail elsewhere.^[41-43] It is well known that there is a strong overlap in substrate specificity and tissue distribution for P-glycoprotein and CYP3A4/5.^[44-46] P-glycoprotein accepts a broad spectrum of structurally and functionally unrelated drugs. It is not clear what determines the substrate specificity of P-glycoprotein. In general, P-glycoprotein substrates are hydrophobic, and/or organic cations at physiological pH, containing one or more aromatic rings, with a molecular weight >400.^[47]

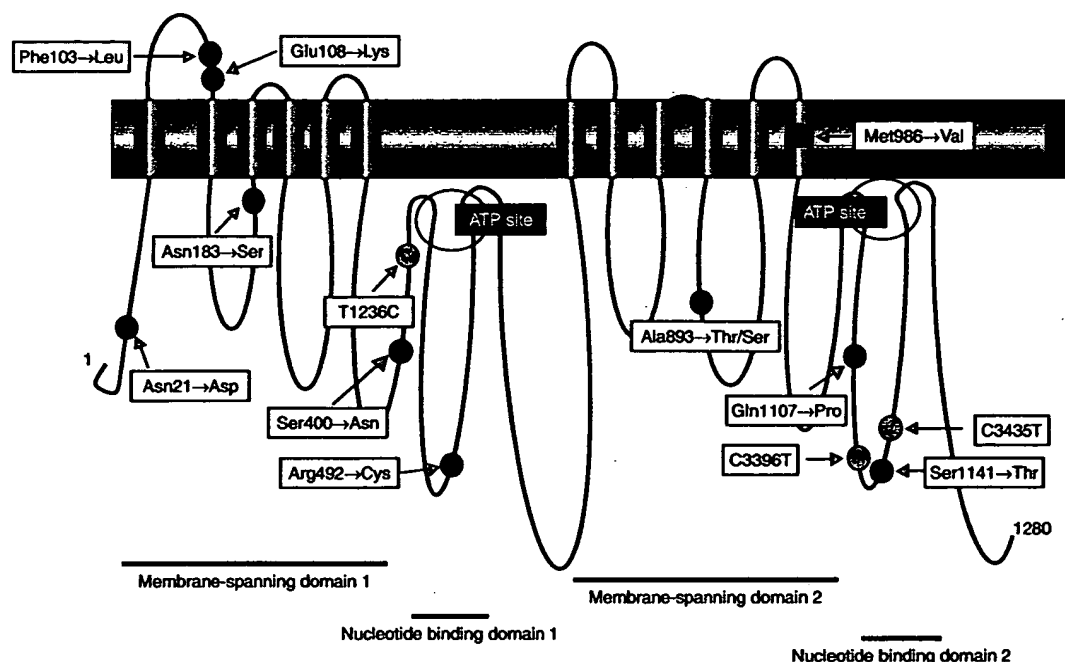


Fig. 1. Schematic representation of P-glycoprotein secondary structure, with known synonymous and non-synonymous nucleotide substitutions. Non-synonymous substitutions are indicated by the corresponding amino acid changes. ATP = adenosine triphosphate.

1.3 Gene Structure and Sites of Polymorphism

The *MDR1* gene is located on chromosome 7 at q21,^[48] with 28 exons encoding a protein of 1280 amino acids. The presence of a highly conserved ATP-binding site in two homologous halves as well as the linker region makes this protein a member of the so-called ATP-Binding Cassette (ABC) transporter superfamily.

Mickley et al.^[49] reported the first evidence of the presence of naturally-occurring polymorphisms in the human *MDR1* gene. They found two SNPs in exon 21 (G2677T) and 24 (G2995A). Subsequently, screening of the entire *MDR1* gene has been undertaken by some laboratories^[9,10,50-52] and, to date, numerous SNPs have been identified (figure 1 and tables I, II and III). Systematic analysis of the entire *MDR1* gene including the promoter region indicated that at least one SNP existed in all DNA samples obtained from healthy Japanese and Caucasian individuals.^[9,10] All of the mutations reported for *MDR1* are SNPs, and some of these are associated with

amino acid substitutions. Whole gene deletion, single nucleotide deletion or changes for aberrant transcription have not been reported. Among non-synonymous mutations, the G→T (G2677T) and G→A (G2677A) transversions at position 2677 in exon 21, which is located on the intracellular side of P-glycoprotein after transmembrane region 10, are associated with an amino acid change from Ala at codon 893 (Ala893) to Ser and Thr, respectively. In contrast, the C→T transversion at 3435 in exon 26 (C3435T) does not change the amino acid sequence. Interestingly, G2677T/A and C3435T are closely linked; >90% of Japanese,^[10] 62% of European American^[52] and 80% of Caucasian German^[53] individuals have these two SNPs simultaneously. The association was also observed in paediatric heart transplant patients.^[5] As regards the linkage disequilibrium, Tang et al.^[54] recently performed a haplotype analysis of the *MDR1* gene in three ethnic Asian groups (Chinese, Malays and Indians) by examining ten intragenic SNPs. Interestingly, three SNPs, located on exons 12 (C1236T), 21 (G2677T/

Table 1. Allelic frequencies of multidrug resistance 1 gene (*MDR1*) variants among different ethnic populations and patient groups. Locations of the intronic single nucleotide polymorphisms (SNPs) are as follows: G-35C (intron 4); G-25T (intron 4); C-139T (intron 6); C-145T (intron 6); T-76A (intron 16); A-137G (intron 17). Amino acid substitutions are indicated with the single-letter code. [Note: Tables II and III are a continuation of table I]

Ethnic background	Subject	A-41aG A/G	C-145G C/G	T-129C T/C	C-4T C/T	G-1A G/A	A61G (21N→D) A/G	G-35C G/C	G-25T G/T	T307C (103F→L) T/C
Asian/Oceanian										
Japanese (n = 100) ⁽¹⁰⁾	Placental cDNA	0.91/0.09	0.97/0.03	0.94/0.06	1.00/0	1.00/0	1.00/0			1.00/0
Japanese (n = 114) ⁽⁵⁶⁾	Volunteers									
Chinese (n = 92-104) ⁽⁵⁴⁾	Neonates (umbilical cords)			0.98/0.02		1.00/0	1.00/0			
Chinese (n = 98-132) ^(64,67)	Volunteers/blood donors									
Filipino (n = 60) ⁽⁵⁶⁾	Volunteers/blood donors									
Indian (n = 61-68) ⁽⁵⁴⁾	Neonates (umbilical cords)			0.98/0.02		1.00/0	1.00/0			
Indian (n = 87) ⁽⁵⁷⁾	Volunteers									
Malay (n = 80-93) ⁽⁵⁴⁾	Neonates (umbilical cords)			0.96/0.04		1.00/0	1.00/0			
Malay (n = 92) ⁽⁵⁷⁾	Volunteers									
Southwest (n = 89) ⁽⁵⁶⁾	Volunteers/blood donors									
Middle East										
Saudi (n = 96) ⁽⁵⁶⁾	Volunteers/blood donors									
African										
Ghananian (n = 172-206) ^(55,56)	Volunteers/blood donors									
Kenyan (n = 80) ⁽⁵⁶⁾	Volunteers/blood donors									
Sudanese (n = 51) ⁽⁵⁶⁾	Volunteers/blood donors									
African American										
African American (n = 23-88) ^(52,56)	Volunteers/blood donors		0.96/0.04			1.00/0	1.00/0			
Caucasian										
Caucasian German (n = 461) ⁽⁵¹⁾	Volunteers					0.91/0.09	0.89/0.11			
Caucasian UK (n = 190) ⁽⁵⁶⁾	Volunteers/blood donors									
Portuguese (n = 100) ⁽⁵⁶⁾	Volunteers/blood donors									
Italian (n = 106) ⁽⁵⁶⁾	Control for Parkinson's disease			0.97/0.03						
Caucasian (n = 537) ⁽⁵¹⁾	Control for renal tumours									

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Table 1. Contd

Ethnic background	Subject	A-41aG A/G	C-145G C/G	T-129C T/C	C-4T C/T	G-1A G/A	A61G (21N→D) A/G	G-35C G/C	G-25T G/T	T307C (103F→L) T/C
Caucasian (n = 37-188) ^[9,52]	Volunteers			0.94/0.06	1.00/0	(0.94-0.98) (0.04-0.06)	(0.90-0.91) (0.09-0.10)	0.99/0.01	0.84/0.16	0.99/0.01
Specific patient groups										
Japanese (n = 17) ^[5]	LDLT recipients	0.79/0.21	0.84/0.08	0.79/0.21	1.00/0	1.00/0	1.00/0			1.00/0
Japanese (n = 68-69) ^[7]	LDLT recipients					1.00/0	1.00/0			
Italian (n = 25) ^[56]	Early onset Parkinson's disease			0.98/0.02						
Italian (n = 70) ^[56]	Late onset Parkinson's disease			0.99/0.01						
Caucasian New Zealand (n = 160) ^[1]	Depressed patients									
Caucasian (n = 124) ^[50]	Renal transplant recipients									
Caucasian (n = 212) ^[51]	Renal epithelial tumours									
LDLT = living donor liver transplantation.										

A) and 26 (C3435T), could account for most of the haplotypes, similar to those reported in European- and African-Americans^[52] and Japanese^[10] populations. They also found inter-ethnic differences in the *MDR1* haplotypes.

On the basis of the assumption that one SNP occurs every 1000 base pairs in a DNA sequence, further novel polymorphisms may be expected. Indeed, we recently observed a novel non-synonymous mutation (Glu108Lys) in Japanese subjects.^[61]

2. Allelic Frequencies of *MDR1* Variants in Different Ethnic/Racial Populations and Specific Patient Groups

2.1 Ethnic/Racial Distribution

The allelic frequency distributions of SNPs in the *MDR1* gene have been reported in various racial populations. Tables I, II and III show the allelic frequencies for *MDR1* variants reported from different ethnic populations and specific patient groups. Among distinct *MDR1* variants, C3435T has been detected in all ethnic populations studied so far (i.e. Caucasians, African Americans and Asians), albeit with considerable ethnic variation in frequencies: the frequency of the C3435 allele has been reported as 43-54% in Caucasians, 34-63% in Asians and 73-90% in Africans. The incidence of C/T and C/C3435 genotypes in the African is much higher than those in other racial populations. At SNP exon 21 G2677T/A, Caucasians (57%) and Japanese (43%) share a similar frequency of the G2677 allele; however, there is a trend toward a lower frequency in the Indian population (34%). An A61G variant is observed only in Caucasians, suggesting it may be ethnic-specific.

Ethnic differences in P-glycoprotein activity have not been widely studied. However, inter-ethnic differences in the distribution of the *MDR1* variants are a possible cause of the inter-ethnic differences in the pharmacokinetics of P-glycoprotein substrate drugs illustrated in the following examples. The oral bioavailability of ciclosporin was significantly lower in Blacks (mean 30.9%) than Whites (39.6%) or Hispanics (42.1%), with no differences in clearance

Table II. (Note: Tables II and III are a continuation of table I)

Ethnic background	Subject	C+139T C/T	C+145T C/T	A548G (183N→S) A/G	G1189A (400S→N) G/A	T1236C T/C	C+44T C/T	C1474T (492R→C) C/T	T-76A T/A	A+137G A/G
Asian/Oceanian										
Japanese (n = 100) ⁽¹⁰⁾	Placental cDNA			1.00/0	1.00/0	0.65/0.35		1.00/0		
Japanese (n = 114) ⁽⁵⁰⁾	Volunteers									
Chinese (n = 92-104) ⁽⁵⁴⁾	Neonates (umbilical cords)					0.69/0.31				
Chinese (n = 98-132) ^(56,57)	Volunteers/blood donors					0.72/0.28				
Filipino (n = 60) ⁽⁵⁸⁾	Volunteers/blood donors									
Indian (n = 61-68) ⁽⁵⁴⁾	Neonates (umbilical cords)					0.61/0.39				
Indian (n = 87) ⁽⁵⁷⁾	Volunteers					0.67/0.33				
Malay (n = 80-93) ⁽⁵⁴⁾	Neonates (umbilical cords)					0.69/0.31				
Malay (n = 92) ⁽⁵⁷⁾	Volunteers					0.66/0.34				
Southwest (n = 89) ⁽⁵⁶⁾	Volunteers/blood donors									
Middle East										
Saudi (n = 96) ⁽⁵⁶⁾	Volunteers/blood donors									
African										
Ghananian (n = 172-206) ^(54,56)	Volunteers/blood donors									
Kenyan (n = 80) ⁽⁵⁶⁾	Volunteers/blood donors									
Sudanese (n = 51) ⁽⁵⁶⁾	Volunteers/blood donors									
African American										
African American (n = 23-89) ^(52,56)	Volunteers/blood donors			1.00/0	1.00/0			1.00/0		
Caucasian										
Caucasian German (n = 461) ⁽⁵¹⁾	Volunteers	0.63/0.37			0.94/0.06	0.41/0.59	0.95/0.05		0.54/0.46	
Caucasian UK (n = 190) ⁽⁵⁶⁾	Volunteers/blood donors									
Portuguese (n = 100) ⁽⁵⁶⁾	Volunteers/blood donors									
Italian (n = 106) ⁽⁵⁶⁾	Control for Parkinson's disease									
Caucasian (n = 537) ⁽⁵¹⁾	Control for renal tumours	0.59/0.41	0.99/0.01	0.99/0.01	(0.94-0.95)/ (0.05-0.06)	0.62/0.38	0.94/0.06	0.99/0.01	0.55/0.45	0.99/0.01
Caucasian (n = 37-188) ^(56,57)	Volunteers									

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Table II. Contd

Ethnic background	Subject	C+139T C/T	C+145T C/T	A548G (183N→S) A/G	G1189A (400S→N) G/A	T1236C T/C	C+44T C/T	C1474T (492R→C) C/T	T-76A T/A	A+137G A/G
Specific patient groups										
Japanese (n = 17) ⁽⁶⁴⁾	LDLT recipients			1.00/0	1.00/0	0.62/0.38		1.00/0		1.00/0
Japanese (n = 68-69) ⁽⁷¹⁾	LDLT recipients	0.37/0.63			1.00/0	0.85/0.35	1.00/0		0.88/0.32	
Italian (n = 25) ⁽⁶²⁾	Early onset Parkinson's disease									
Italian (n = 70) ⁽⁶²⁾	Late onset Parkinson's disease									
Caucasian New Zealand (n = 160) ⁽⁷¹⁾	Depressed patients									
Caucasian (n = 124) ⁽⁶²⁾	Renal transplant recipients									
Caucasian (n = 212) ⁽⁶²⁾	Renal epithelial tumours									

LDLT = living donor liver transplantation.

or the volume of distribution at steady state.^[62] Since intestinal P-glycoprotein activity is the major determinant of the bioavailability of ciclosporin, patients with a higher level of intestinal P-glycoprotein may have lower bioavailability and whole blood concentrations, and vice versa.^[63] Inter-ethnic differences in bioavailability and interaction profiles of tacrolimus have also been reported.^[64-66] As described above, the frequency of the C/C3435 genotype is higher in Africans than in other racial populations. Recently, Schaeffeler and colleagues^[58] speculated that the reason for the lower bioavailability of ciclosporin in Africans than in Caucasians is an increased frequency of the C/C genotype in Africans, based on their experimental findings that individuals homozygous for the T3435 allele have on average substantially lower intestinal P-glycoprotein levels than those homozygous for the C3435 allele.^[9] The high frequency of the C3435 allele in Africans may also explain the high incidence of resistance and more aggressive tumours, such as breast cancer, in individuals of African origin.^[56,67] These data highlight the need to consider inter-ethnic variability before extrapolating pharmacokinetic data, including drug interaction profiles, obtained in one ethnic group to another.

2.2 Specific Patient Groups

Since P-glycoprotein may have a role as a protective barrier against a wide variety of substrates as well as the environment, the allelic frequency of *MDR1* variants is expected to differ among patients with various diseases, as has been seen among different racial populations.

Based on the functional role of P-glycoprotein as a neuroprotective barrier, altered P-glycoprotein expression or function in brain capillaries, partially due to polymorphism of the *MDR1* gene, could affect the uptake of neurotoxic xenobiotics, thereby modulating interindividual susceptibility to neurological disorders such as Parkinson's disease. To test this hypothesis, Furuno et al.^[59] compared allelic frequencies of three mutations (C3435T, G2677T/A, and T-129C) between 95 Italian patients with Parkinson's disease and 106 non-Parkin-

Table III. [Note: Table III is a continuation of tables I and II]

Ethnic background	Subject	C2650T C/T	G2677T/A (893A→S,T)(986M→V) G/T/A	A2956G A/G	A3320C (1107Q→P) A/C	C3396T C/T	T3421A (1141S→T) T/A	C3435T C/T	G4030C (3'end) G/C	A4036G (3'end) A/G
Asian/Oceanian										
Japanese (n = 100) ⁽¹⁰⁾	Placental cDNA		0.43/0.39/ 0.18	0.99/0.01	1.00/0	1.00/0	1.00/0	0.59/0.42	0.99/0.01	0.69/0.31
Japanese (n = 114) ⁽⁵³⁾	Volunteers							0.81/0.39		
Chinese (n = 92–104) ⁽⁵⁴⁾	Neonates (umbilical cords)		0.51/0.44/ 0.06					0.60/0.40		
Chinese (n = 96–132) ^(54,57)	Volunteers/blood donors		0.38/0.50/ 0.13					(0.47–0.53)/ (0.47–0.53)		
Filipino (n = 60) ⁽⁵⁸⁾	Volunteers/blood donors		0.34/0.62/ 0.04					0.59/0.41		
Indian (n = 61–68) ⁽⁵⁴⁾	Neonates (umbilical cords)		0.33/0.60/ 0.07					0.40/0.60		
Indian (n = 87) ⁽⁵⁷⁾	Volunteers							0.37/0.63		
Malay (n = 80–83) ⁽⁵⁴⁾	Neonates (umbilical cords)		0.57/0.38/ 0.07					0.63/0.37		
Malay (n = 92) ⁽⁵⁷⁾	Volunteers		0.53/0.44/ 0.03					0.49/0.51		
Southwest (n = 89) ⁽⁵⁸⁾	Volunteers/blood donors							0.34/0.66		
Middle East										
Saudi (n = 96) ⁽⁵⁸⁾	Volunteers/blood donors							0.55/0.45		
African										
Ghananian										
(n = 172–206) ^(54,58)	Volunteers/blood donors							(0.83–0.90)/ (0.10–0.17)		
Kenyan (n = 80) ⁽⁵⁸⁾	Volunteers/blood donors							0.83/0.17		
Sudanese (n = 51) ⁽⁵⁸⁾	Volunteers/blood donors							0.73/0.27		
African American										
(n = 23–88) ^(52,56)	Volunteers/blood donors	1.00/0					0.96/0.04	0.84/0.16		
Caucasian										
Caucasian German										
(n = 461) ⁽⁵¹⁾	Volunteers		0.57/0.42/ 0.02		0.99/0.01			0.46/0.54		
Caucasian UK (n = 190) ⁽⁵⁴⁾	Volunteers/blood donors							0.48/0.52		
Portuguese (n = 100) ⁽⁵⁴⁾	Volunteers/blood donors							0.43/0.57		
Italian (n = 106) ⁽⁵⁹⁾	Control for Parkinson's disease		0.56/0.41/ 0.03					0.54/0.46		
Caucasian (n = 537) ⁽³⁾	Control for renal tumours							0.50/0.50		

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Table III. Contd

Ethnic background	Subject	C2650T C/T	G2677T/A (893A→S,T)(886M→V) G/T/A	A2956G (1107Q→P) A/C	C3398T C/T	T3421A (1141S→T) T/A	C3435T C/T	G4030C (3'end) G/C	A4036G (3'end) A/G
Caucasian (n = 37-188) ^[6,24]	Volunteers	0.97/0.03			0.99/0.01	1.00/0	0.52/0.48		
Specific patient groups									
Japanese (n = 17) ^[6]	LDLT recipients	1.00/0	0.59/0.32/ 0.09	1.00/0	1.00/0	1.00/0	0.53/0.47	1.00/0	0.62/0.38
Japanese (n = 68-69) ^[7]	LDLT recipients		0.45/0.44/ 0.12				0.56/0.44		
Italian (n = 25) ^[6a]	Early onset Parkinson's disease		0.50/0.48/ 0.02				0.42/0.58		
Italian (n = 70) ^[6a]	Late onset Parkinson's disease		0.53/0.44/ 0.04				0.49/0.51		
Caucasian New Zealand (n = 160) ^[1]	Depressed patients						0.53/0.47		
Caucasian (n = 124) ^[6a]	Renal transplant recipients						0.46/0.55		
Caucasian (n = 212) ^[3]	Renal epithelial tumours						0.42/0.58		
LDLT = living donor liver transplantation.									

son's disease, non-medicated controls. They divided the 95 patients into two groups according to the age at onset of the disease; an early onset group (n = 25, onset age ≤45 years) and a late onset group (n = 70, onset age >45 years). Although the differences did not reach statistical significance, the frequencies of T3435 and T2677 were highest in the early onset Parkinson's disease group, second highest in the late onset group, and lowest in the control group. Siegmund et al.^[3] also compared the frequencies of allelotypes and genotypes of C3435T between patients with renal epithelial tumours and healthy control subjects. They observed a significant disequilibrium with respect to a higher T3435 prevalence in patients.

Ulcerative colitis in humans has histological features resembling a form of colitis developed in *mdr1* knockout mice.^[68,69] Since ulcerative colitis in *mdr1a*^{-/-} mice can be prevented by antibiotics and since the *mdr1a*^{-/-} mice are immunologically normal, functional defects of the intestinal epithelial barrier due to the lack of P-glycoprotein expression are possible reasons for the pathogenesis of colitis.^[68] Schwab et al.^[70] tested whether C3435T polymorphism, which is associated with a lower intestinal P-glycoprotein expression,^[9] predisposes one to the development of ulcerative colitis by comparing allele frequencies and genotype distribution of C3435T between 149 patients with ulcerative colitis and sex-matched healthy controls. They found significantly increased frequencies of the T3435 allele and homozygotes for the T allele in patients with ulcerative colitis compared with controls, and concluded that impairment of barrier function in T3435 subjects could render this genotype more susceptible to the development of ulcerative colitis.

Some SNPs have been identified in the 5'-untranslated region (5'-UTR) of the *MDR1* gene. Interestingly, these mutations are frequently observed in patients with haematological malignancies^[71] or osteosarcomas.^[72] Several studies have shown that certain specific nucleotide sequences, including SP1 and AP1 binding sites, are important in controlling the level of *MDR1* expression.^[73-75] Rund et al.^[71] have identified a T→C transversion at position +8

(relative to the transcription start site) localising near (one base pair downstream) the initiator sequence (-6 to +11) required for proper transcription initiation. They also found that this mutation was present in patients with acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia at three times the frequency that it was found in normal subjects. A T→C transversion at position +8 is identical to the T-129C mutation.

3. Polymorphisms and Expression of P-Glycoprotein in Various Human Tissues

P-glycoprotein levels are known to show significant interindividual variability, with 2- to 8-fold variations found in intestinal biopsies from patients and healthy volunteers.^[63] Among various mutations in the *MDR1* gene, certain variants have recently been reported to be associated with a change in expression levels of P-glycoprotein in human tissues. Table IV summarises the effect of different *MDR1* genotypes on the expression level of P-glycoprotein in various human tissues, together with *in vivo* pharmacokinetic and pharmacodynamic changes of P-glycoprotein substrates.

Using an immunochemical approach to quantify P-glycoprotein content, Hoffmeyer et al.^[9] first reported that C3435T was associated with a significantly reduced intestinal P-glycoprotein content in subjects with the T/T genotype (i.e. the homozygote for the mutant T allele) in comparison with subjects homozygous for the C allele. In the human placenta, Tanabe et al.^[10] found that individuals having the C-129 allele showed significantly lower P-glycoprotein levels than those having the T-129 allele, while such a significant correlation was not observed for the C3435T allele, as measured by Western blot analysis. T-129C is located seven basepairs downstream from the transcription initiation site (A⁺1TTCGAGTAG). In their study, G267T/A also correlated with the level of P-glycoprotein expression, but not significantly. In the human heart (left ventricular samples), a reduced expression, but not statistically significant, in samples containing the

T/T genotype of the C3435T mutation was reported.^[84]

P-glycoprotein and *MDR1* mRNA are also expressed in various leucocyte lineages with the highest level of expression in CD56⁺ natural killer cells, followed by CD4⁺, CD15⁺, CD19⁺ and CD14⁺ cells.^[85,86] Hitzl et al.^[82] studied *MDR1* mRNA expression in leucocytes of healthy individuals with different genotypes at position 3435 of the human *MDR1* gene and found that the *MDR1* mRNA level was lowest in the T/T population, intermediate in heterozygous subjects and highest in the C/C group. Reduced P-glycoprotein activity was also found in natural killer cells from healthy individuals having the T/T genotype at position 3435 in comparison with subjects homozygous for the C allele.^[79,82] Fellay et al.^[2] have quantified by real-time PCR *MDR1* transcripts in peripheral blood mononuclear cells from 59 HIV-1-infected patients, and showed an association between the C3435T T/T genotype and a lower level of *MDR1* expression (arbitrary unit, median 1.87) compared with the C/T genotype (2.36) and C/C genotype (2.79). They also confirmed this association by fluorescence-activated cell-sorter analysis of P-glycoprotein expression in peripheral blood mononuclear cells; the correlation coefficient between transcript and protein expression was $r = 0.58$ ($p < 0.0006$). Siegmund et al.^[3] have also indicated an association of the T3435 allele and lower P-glycoprotein expression in non-cancerous renal tissues.

In contrast to these observations, Nakamura et al.^[8] have indicated that the T3435 allele was associated with increased *MDR1* mRNA expression in human duodenal samples. Similarly, Illmer et al.^[4] showed a lower *MDR1* expression in blast samples obtained from patients with AML whose *MDR1* genotypes were homozygous for the wild-type allele at all three gene loci investigated (i.e. exons 12, 21, and 26). However, Goto et al.^[7] and Siegmund et al.^[53] have recently reported that intestinal or duodenal *MDR1* mRNA levels were not influenced by C3435T polymorphism. The latter authors have also determined immunoreactive duodenal P-glycoprotein expression and observed the same results for

Table IV. Effects of genetic polymorphism of *MDR1* on the expression of P-glycoprotein in human tissues and *in vivo* pharmacokinetics and pharmacodynamics of P-glycoprotein substrates

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	<i>In vitro</i> efflux	Expression level
Hoffmeyer et al. ^[9]	C3435T	Digoxin	HV/human duodenum	T/T > C/C (C_{max})		C/C > C/T > T/T (protein concentration)
Kerb et al. ^[76]	C3435T	Phenytoin	HV	T/T > C/C (plasma concentration)		
Kurata et al. ^[11]	C3435T, G2677T	Digoxin	HV	M/M > W/M > W/W in both loci (F), W/W > W/M > M/M in both loci (CL_R , CL_{sec})		
Min and Ellingrod ^[77]	C3435T	Cyclosporin	HV	(T/T + C/T) > C/C (C_{max} and AUC, but not significantly different)		
Johne et al. ^[78]	C3435T, G2677T	Digoxin	HV	TT > TC > CC (C3435T, AUC and C_{max}). Haplotype 12 > haplotype 11 (AUC, C_{max})		
von Ahsen et al. ^[60]	C3435T	Cyclosporin	RTR	C/C = C/T = T/T (dose-adjusted C_{min} and rejection incidence)		
Chowbay et al. ^[67]	C1236T, G2677T, C3435T	Cyclosporin	HTR	TT-TT-TT > CT-GT-CT > CC-GG-CC. T-T-T > C-G-C haplotype. (AUC, C_{max} , C_{min})		
Drescher et al. ^[79]	C3435T	Fexofenadine, rhodamine 123	HV/CD56+ cell	C/C = T/T (AUC)	C/C > C/T > T/T	

Continued next page

Table IV. Contd

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	In vitro efflux	Expression level
Goto et al. ^[7]	C-139T, C1236T, T-78A, G2677T/A, C3435T	Tacrolimus	LDLTR/human intestine	No significant effect of SNPs on tacrolimus concentration/dose ratio		No effect (mRNA level)
Siegmund et al. ^[83]	C3435T, G2677T/A	Talinolol	HV/human duodenum	W/W = W/M = M/M in both loci (AUC)		No significant effect (protein and mRNA levels)
Goh et al. ^[84]	C3435T	Docetaxel	Cancer patients	C/C = C/T = T/T (CL)		
Kim et al. ^[82]	G2677T *2 allele	Fexofenadine, digoxin	HV/NIH-3T3 GP+E86 cells	*1/*1 > *1/*2 > *2/*2 (AUC)	MDR1-Ser893 > MDR1-Ala893	
Sakaeda et al. ^[86]	C3435T	Digoxin	HV	C/C > subjects with the T allele (i.e. C/T and T/T) [AUC ₀₋₄]		
Roberts et al. ^[11]	C3435T	Nortriptyline	Depressed patients	T/T > C/T > C/C (frequency of drug-induced postural hypotension)		C/C > C/T > T/T (protein and mRNA levels)
Fellay et al. ^[2]	C3435T	Neftinavir, efavirenz	HIV-1-infected patients/PBMC	T/T > C/T > C/C (CD4+ cell count and recovery of naive CD4+ cells). C/C > C/T > T/T (C _{min})		
Zheng et al. ^[8]	C3435T, G2677T	Corticosteroids	Pediatric heart transplant recipients	W/W > W/M > M/M (duration of corticosteroid therapy)		C/T > T/T > C/C (1236), G/T > T/T > G/G (2677), C/T > T/T > C/C (3435) [mRNA level]
Illmer et al. ^[4]	C1236T, G2677T, C3435T		AML patients/biast samples	W/M > M/M > W/M in all 3 loci (overall survival). W/W > M/M > W/M in all 3 loci (probability of relapse)		

Continued next page

Table IV. Contd

Study	Polymorphism	Substrate	Subject/material	Pharmacodynamic-pharmacokinetic outcome	In vitro efflux	Expression level
Potocnik et al. ^[81]	T-129C, IVS1-81delG		Tumour samples (colorectal adenocarcinoma)	Association with lymphoid infiltration		W > M (protein level)
Yamauchi et al. ^[82]	G2677T/A	Tacrolimus	LDLTR	Positive predictor of drug-induced neurotoxicity		
Siegmund et al. ^[83]	C3435T		Healthy control and non-CCRCC patients/non-cancerous renal tissues	T allele as a risk factor		C/C > T/T (protein level)
Schwab et al. ^[70]	C3435T		Inflammatory bowel disease (Crohn's disease and ulcerative colitis) patients	T > C allele, T/T genotype > other types (allelic frequency in patients with ulcerative colitis)		
Hlitzl et al. ^[82]	C3435T	Rhodamine 123	CD56+ cell		C/C > C/T > T/T	C/C > C/T > T/T (mRNA level)
Calado et al. ^[83]	T-129C, G2677T, C3435T	Rhodamine 123	CD34+ cells		W/W = W/M = M/M	
Tanabe et al. ^[10]	T-129C, G2677T/A, C3435T		Human placenta			T/T > T/C (-128), W/W > W/M > M/M (2677), C/C = C/T = T/T (3435) [protein level]
Nakamura et al. ^[84]	C3435T		Human duodenum			T/T > C/T > C/C (mRNA level)
Meissner et al. ^[84]	C3435T		Human heart			Reduced in T/T samples (protein and mRNA levels)

AML = acute myeloid leukaemia; AUC = area under the plasma concentration-time curve; CCRCC = clear cell renal cell carcinoma; CL_R = renal clearance; CL_{sec} = renal secretory clearance; C_{max} = peak plasma concentration; C_{min} = trough plasma concentration; F = bioavailability; HTR = heart transplant recipients; HV = healthy volunteers; LDLTR = living donor liver transplantation recipients; M = mutant allele; mRNA = messenger RNA; PBMC = peripheral blood mononuclear cells; RTR = renal transplant recipients; SNP = single nucleotide polymorphism; W = wild-type allele.

mRNA levels.^[53] Thus, the collected evidence indicates that the contribution of the *MDR1* variants to expression (both at protein and mRNA levels) is still controversial.

4. Impact of Polymorphisms on Pharmacotherapy

4.1 Pharmacokinetic Consequences

To date, polymorphisms of the *MDR1* gene that alter *in vivo* transport activity have been focused on: the silent mutation in exon 26 (C3435T) and the non-synonymous mutation in exon 21 (G2677T/A).

Subjects with the T/T genotype at position 3435 had higher steady-state plasma concentrations after oral administration of digoxin in comparison with the C/C subjects.^[9,78] Similar results were observed by Kurata et al.,^[11] who showed that the mean absolute bioavailability (estimated from oral and intravenous administrations) of digoxin was significantly higher in 2677TT/3435TT subjects (homozygotes for thymine at both positions 2677 and 3435) than 2677GG/3435CC subjects in a gene dose-dependent manner, in that maximum bioavailability was observed in homozygotes for the mutant allele (mean, 87.1%) > heterozygotes (80.9%) > homozygotes for the wild-type allele (67.6%). They also indicated that the renal clearance of digoxin was almost 32% lower in 2677TT/3435TT subjects than in 2677GG/3435CC subjects, with 2677GT/3435CT subjects having an intermediate value. These results suggest that reductions in the intestinal secretion of digoxin into the gut lumen and renal excretion into the urine occur simultaneously in subjects with SNPs.

The histamine H₁ receptor antagonist fexofenadine, which is used for the treatment of seasonal allergic rhinitis and chronic idiopathic urticaria, is also a P-glycoprotein substrate. Kim et al.^[52] demonstrated that the *2 allele was associated with differences in fexofenadine concentrations, with the area under the plasma concentration-time curve (AUC) being almost 40% greater in *1/*1 subjects compared with *2/*2 subjects, with *1/*2 heterozygotes having an intermediate value, suggesting en-

hanced *in vivo* P-glycoprotein activity among subjects with the *MDR1* *2 allele. In their study, the *2 allele was defined as a haplotype in which three SNPs at different polymorphic sites (T1236, T2677 and T3435) occurred simultaneously. However, they reported that fexofenadine is also a good substrate for organic anion transporting polypeptide (OATP).^[87,88] In contrast, Drescher et al.^[79] did not find any significant differences in fexofenadine disposition between subjects homozygous for the C allele and T allele at position 3435. Siegmund et al.^[53] also did not find a significant influence of three *MDR1* variants (C3435T and G2677T/A) on talinolol disposition.

Because of a lack of metabolic biotransformation, digoxin is often used as a probe drug for pharmacogenetic testing (i.e. *in vivo* phenotype-genotype relationship studies) of the *MDR1* gene polymorphism. Sakaeda et al.^[55] studied the relationship between the *MDR1* genotype and the pharmacokinetics of digoxin after a single oral administration in healthy subjects. They found that the AUC₄ of digoxin was significantly lower in subjects with the T/T3435 genotype than in C/C3435 subjects. Their observations are in line with a finding by Kim et al.,^[52] but in contrast to the findings of Hoffmeyer et al.^[9] and Kurata et al.^[11]

One study has investigated the relationship between *MDR1* polymorphisms and the pharmacokinetics of oral ciclosporin in healthy subjects. Although the peak concentration and AUC of ciclosporin in the C/T3435 and T/T3435 subjects were 15% and 22% larger than those in C/C3435 subjects, differences in these values did not reach statistical significance.^[77]

Functional consequences of *MDR1* polymorphism have also been investigated in two *in vitro* studies. In *in vitro* experiments conducted by Kim et al.^[52] with cultured cells expressing MDR1-Ala893 (G2677) and MDR1-Ser893 (T2677) revealed that the Ser893 variant transporter resulted in a 47% lower intracellular digoxin concentration than did the Ala893 variant. Based on these results, they concluded that Ser893 variant-containing cells exhibit enhanced efflux characteristics compared with

those cells in which Ala893 was expressed. Kimchi-Sarfaty et al.^[89] also investigated functional consequences of *MDR1* polymorphisms (Asn21Asp, Phe103Leu, Ser400Asn, Ala893Ser, and Ala998Thr) using a vaccinia virus-based transient expression system by two approaches; cell surface localisation and transport function. In contrast to the findings by Kim et al.,^[52] they found that cell surface expression and transport capabilities were not substantially affected by any of the polymorphisms tested.

Taking all these findings into consideration, published observations, even when made using the same probe drug and even among the same racial group, are conflicting. The question arises as to why the contribution of C3435T and/or G2677T/A mutations to the pharmacokinetics of digoxin and fexofenadine differs among reports. Discussing possible reasons for this discrepancy will be useful for future studies of the involvement of polymorphisms of *MDR1*, as well as other drug transporters, in *in vivo* transport activity.

Both digoxin and fexofenadine are transported across cells by the OATPs, which are also expressed in various human tissues such as liver, intestine, and kidney. Although the intestinal transport mechanisms responsible for fexofenadine uptake have not yet been defined, fexofenadine has been shown to be a substrate of human OATP-A.^[87,88] Similarly, digoxin was reported to be a substrate of liver-specific OATP8, another member of the OATP family.^[90] In addition to the *MDR1* gene, the *OATP-C* and *OATP8* genes exhibit genetic variability.^[91,92] Although the effects of *OATP8* variants have not yet been elucidated, certain mutations in the *OATP-C* gene could alter the *in vivo* pharmacokinetics of a clinically used drug.^[93] Thus, it is possible that other transport mechanisms apart from those involving P-glycoprotein contribute to the variations in digoxin and fexofenadine pharmacokinetics in humans.

Grapefruit is known to inhibit the intestinal metabolism of numerous drugs, including terfenadine, saquinavir, ciclosporin, triazolam and nisoldipine, by inhibiting CYP3A enzymes, resulting in elevated drug bioavailability and then serum concentra-

tions.^[94] Recently, a new mechanism for the drug-grapefruit juice interaction has been reported; the bioavailability and serum concentrations of fexofenadine were reduced when grapefruit juice was taken.^[95] In the intestine, P-glycoprotein and OATPs are located on the luminal membrane of the enterocyte, but they have opposite vectors for efflux back into the bowel and for uptake into the portal circulation, respectively.^[95] Although the specific OATP member(s) responsible for the fexofenadine-grapefruit juice interaction has not been elucidated, OATP-B was recently identified as an OATP member localised at the apical membrane of intestinal epithelial cells in humans.^[96] In addition to OATP-B, OATP-D and OATP-E are reported to be expressed in the human small intestine.^[97] Since grapefruit juice is a more potent *in vitro* inhibitor of OATP than of P-glycoprotein activities,^[95] the entry of fexofenadine from the intestinal lumen to blood may be inhibited by grapefruit juice, resulting in a reduced bioavailability. As grapefruit is able to inhibit P-glycoprotein-mediated drug efflux when present in sufficient concentrations,^[95,98,99] the net bioavailability of fexofenadine will depend on the relative contribution of both efflux and uptake mechanisms. Indeed, in an interaction study with grapefruit juice, non-significant but moderate changes in digoxin pharmacokinetics were observed.^[99] These findings clearly indicate that the intestinal transport of fexofenadine is determined by at least two drug transporters (P-glycoprotein and OATPs). Thus, multi-transporter-mediated drug transport with genetic variability needs to be considered when evaluating transport activities in the human body. It is clear that the identification of specific probe substrates and inhibitors for P-glycoprotein is required to elucidate the *in vivo* effect of *MDR1* polymorphisms on pharmacotherapy.

The possibility of the existence of functional unobserved SNPs cannot be excluded. As described in section 2, three SNPs, C1236T, G2677T/A and C3435T, have been haplotyped.^[10,13,52,54] Recently, Tang et al.^[54] demonstrated linkage disequilibrium between the different pairs of these SNPs and speculated on unobserved causal SNP(s) near position

3435, which might provide a plausible explanation for the conflicting findings among reports. However, based on the collective evidence from previous systematic analyses of the entire *MDR1* gene,^[9,10,50,51] it is anticipated that such functional unobserved mutation(s) would not be localised to the coding region. Nevertheless, haplotype-based approaches, which take into consideration the combination of SNPs present in one allele, are expected to offer greater ability to predict changes in phenotype than SNP-based approaches.^[100,101] Johnne et al.^[78] recently reported that the analysis of *MDR1* haplotypes is superior to an unphased SNP analysis for predicting *MDR1* phenotype. They speculated that haplotype 12 (i.e. 2677GT/3435TT) is a key genotype to describe interindividual differences in the pharmacokinetics of substrate drugs and account for divergent results among reports.

There are some reports about specific mechanisms of upregulation of *MDR1* transcription. Nakayama et al.^[102] reported hypomethylation of the *MDR1* promoter as a predictive factor for *MDR1* upregulation in patients with AML, and Mickley et al.^[103] showed gene rearrangements as causative events for *MDR1* expression. DNA methylation is one plausible regulator of gene expression. Mammalian DNA is heavily methylated at cytosine residues within CpG dinucleotides, with 60–80% of such residues being methylated.^[104,105] Histone acetylation is associated with an increase in the accessibility of DNA to transcriptional machinery. The presence or absence of methylation at CpG sites (i.e. epigenetic mechanisms) is closely associated with transcriptional activation of the *MDR1* gene in various cultured cell lines and human tumour samples.^[102,106–109] However, unfortunately, there are currently no data about differential *MDR1* gene regulation in normal tissues.

4.2 Pharmacodynamic Consequences

Besides the direct effect of genetic polymorphism on the pharmacokinetic profiles of substrate drugs, which may be responsible for the intended therapeutic effect and/or toxicity, the association

between genetic variations and clinical outcomes remains largely unexplored.

As described previously, Fellay et al.^[2] have studied the association between response to antiretroviral treatment and allelic variants of *MDR1* in 123 HIV-1-infected patients who were treated with efavirenz or nelfinavir. They found an association of T3435 with lower P-glycoprotein expression levels in peripheral blood mononuclear cells and a better response to anti-HIV-1 drugs as determined by an increased CD4+ cell count. CD4+ T-lymphocytes are the major cellular target of HIV-1 protease inhibitors.^[110] A more pronounced P-glycoprotein activity in subjects with the C/C genotype compared with T/T subjects could limit intracellular concentrations of these drugs, thereby limiting their therapeutic efficacy.^[2,82] They also reported the surprising finding that the T allele was associated with lower concentrations of nelfinavir and efavirenz in plasma, even with a low expression of P-glycoprotein in peripheral blood mononuclear cells. In order to address this paradox, they explored the following two hypotheses for a reduction of plasma drug concentrations: overexpression of other transporters with affinity for antiretroviral drugs and/or induction of CYP3A as compensatory adaptations to low concentrations of P-glycoprotein, as have been observed in *mdr1*-knockout mice.^[111,112] However, they could not identify such compensatory mechanisms by analysis of transcription levels of *ABCC1* (MRP1) and *ABCC2* (MRP2), which encode for multidrug-resistant protein with affinity for antiretroviral agents,^[113,114] or by assessment of CYP3A activity using midazolam as a probe drug.^[115] The results about *MDR1* polymorphisms in their study raise another issue, in that the plasma concentrations of efavirenz, which is not a known substrate of P-glycoprotein, had a similar distribution pattern (i.e. lower concentrations in patients with the T allele) to those of nelfinavir, which is a well defined substrate of P-glycoprotein.^[113] Interestingly, in addition to genetic variation in *MDR1*, *CYP2D6* genotypic status was a weak predictor for the interindividual variations in plasma concentrations of the two study drugs. Indeed, patients having the *CYP2D6* allele

associated with a poor metaboliser phenotype had higher concentrations in plasma of both drugs than did patients with a *CYP2D6* extensive metaboliser genotype when the patients' *MDR1* genotype was matched. Although *CYP2D6* has not been reported to be a predominant enzyme for the metabolism of these drugs, the *CYP2D6* genotype may be a partial explanation for the paradoxical results.

Roberts et al.^[1] evaluated the association between drug-induced adverse effects and *MDR1* gene polymorphism. Postural hypotension is a problematic adverse effect of tricyclic antidepressant medication that occurs in 10–50% of patients at therapeutic dosages, and is characterised by dizziness, palpitations and headache. They found that patients homozygous for the T allele at exon 26 (C3435T) had an increased risk of nortriptyline-induced postural hypotension, although neither the nortriptyline dose nor blood concentrations of drug differed significantly by genotype group.

Neurotoxicity is one of the most important and serious adverse effects of tacrolimus. Yamauchi et al.^[6] have recently evaluated the correlation of *MDR1* gene polymorphism with tacrolimus-induced neurotoxicity (e.g. convulsions, tremor and leukoencephalopathy) in patients after living-related donor liver transplantation, and found that a high tacrolimus concentration, liver dysfunction and a mutation at position 2677 in exon 21 (i.e. the T2677 allele) were positive predictors of toxicity by a stepwise discriminant function analysis. Since P-glycoprotein regulates the distribution of substrate drugs (e.g. nortriptyline and tacrolimus) through the blood-brain barrier into the brain, a reduction in P-glycoprotein function and expression could lead to an abnormal accumulation of prescribed drugs in the brain. In living-donor liver transplantation, intestinal *MDR1* expression is also found to predict both tacrolimus pharmacokinetics and patient survival.^[116] In this study, G2677T/A was a positive predictor for the development of tacrolimus neurotoxicity, whereas C3435T negatively contributed to toxicity, suggesting functional differences between the two SNPs.

Although these two SNPs are in tight linkage disequilibrium, their functional linkage to other SNPs is not identical. One systematic analysis of entire placental cDNA has indicated that heterozygous samples for the T-129C allele also had a mutant 2677T/A allele; however, an association between T-129C and C3435T was not observed.^[10] Although the T-129C polymorphism is not located on known regulatory elements, it was shown to be associated with a lower P-glycoprotein expression in placenta.^[10] Thus, whether the major three polymorphisms (i.e. C1236T, G2677T/A and C3435T) are functionally linked to polymorphic positions at regulatory sites of the *MDR1* promoter is of interest.

Corticosteroids are frequently prescribed with tacrolimus for the purpose of immunosuppression in transplant recipients. Zheng et al.^[5] recently demonstrated an association between polymorphisms of the *MDR1* gene and corticosteroid weaning in 65 paediatric heart transplant patients, and indicated that homozygotes for the C3435 allele or G2677 allele required longer (at 1 year after transplantation) prednisone therapy than did patients having SNPs. Patients with the C/C3435 genotype and the G/G2677 genotype may require more aggressive alternative therapy if corticosteroids are going to be deleted from the immunosuppressive regimen.^[5]

MDR1 gene polymorphisms are also reported to affect the outcome of therapy in patients with AML. Illmer et al.^[4] compared the clinical course of AML treatment among patients with various *MDR1* genotypes, and demonstrated that patients homozygous for the wild-type allele at any locus investigated (exons 12, 21 and 26) exhibited a significantly decreased overall survival with a higher probability of relapse. Theoretically, a reduced intracellular concentration of anticancer drugs attributable to the action of P-glycoprotein (i.e. efflux transport of drugs to the outside of cells) in AML blasts may be related to resistant disease and failure of AML therapy. The patients were uniformly treated with anticancer drugs^[4,117] that are P-glycoprotein substrates, including etoposide,^[128] mitoxantrone^[118] and daunorubicin.^[119] Although they did not determine

whether the association between *MDR1* polymorphism and survival of AML patients was attributable to altered P-glycoprotein-mediated drug pharmacokinetics, increased clearance of these drugs in patients homozygous for the wild-type allele at the three loci might explain these findings. However, they showed a clear correlation between homozygosity for the wild-type allele(s) and lower *MDR1* expression in blast samples. Taken together, these findings, as well as those of Fellay et al.,^[2] raise the possibility of differential gene regulation in different tissues, especially between normal tissues and leukaemic blasts. Nevertheless, a number of studies have indicated that overexpression of P-glycoprotein caused by *MDR1* gene amplification can be applied as a prognostic marker in certain diseases, such as leukaemia or ovarian cancer; high *MDR1* levels being indicative of a poor prognosis.^[120-123]

Since P-glycoprotein is expressed in lymphocytes,^[124,125] it has been hypothesised that overexpression of P-glycoprotein may be one of the reasons for acute and chronic rejection episodes despite adequate ciclosporin concentrations in blood.^[126,127] However, von Ahsen et al.^[60] reported no remarkable differences between stable renal transplant recipients with and without the C3435T mutation regarding renal function and the incidence of acute rejection as determined by biopsy during ciclosporin immunosuppression therapy. They also observed no differences in dose-adjusted ciclosporin trough concentrations between the two genotype groups. By contrast, Chowbay et al.^[57] recently investigated the influence of *MDR1* polymorphisms on the pharmacokinetics of oral ciclosporin in 14 stable heart transplant patients. They focused on the haplotype of C1236T, G2677T/A and C3435T polymorphisms. Of the four major haplotypes identified in Asian populations (C-A-C, C-G-C, T-G-C and T-T-T), the T-T-T haplotype was frequently observed in all three racial populations (50% for Indians, 41% for Chinese and 37% for Malays). They also indicated that patients with the CC-GG-CC genotypes (C-G-C haplotype) had lower ciclosporin exposure, as determined from AUC₄, AUC₁₂ and peak concentration, compared with pa-

tients with the TT-TT-TT genotypes (T-T-T haplotype), and suggested that Indians, in whom the T-T-T haplotype frequency was highest, may require a ciclosporin dosage regimen different from that in Chinese and Malays.

4.3 Interaction Profiles

In contrast to the extensive analysis of the pharmacological and physiological role of P-glycoprotein, surprisingly little has been reported regarding *MDR1* polymorphisms and drug-drug interaction profiles. Hoffmeyer et al.^[9] first provided an interesting finding that the mean of the rifampicin (rifampin)-induced digoxin concentration of the C3435 population was lower than that of the T3435 population. Several studies have reported a digoxin-clarithromycin interaction, which is characterised by a significant elevation of the steady-state plasma concentration of digoxin.^[128-131] Recently, Kurata et al.^[11] have provided evidence that the oral bioavailability of digoxin during administration of clarithromycin was significantly increased in 2677GG/3435CC subjects, whereas no such significant change was observed in subjects with the *MDR1* gene SNPs. Although the molecular mechanism behind the inhibition of P-glycoprotein by clarithromycin is not precisely known, the most plausible reason for the remarkable changes in oral bioavailability in 2677GG/3435CC subjects would be that they have relatively greater amounts of intestinal P-glycoprotein to be inhibited by clarithromycin. Similar genotype-specific drug interactions have been reported for a known polymorphic metabolising protein, CYP2C19, including moclobemide-omeprazole,^[132] diazepam-omeprazole^[133] and proguanil-omeprazole^[134] interactions.

It is highly likely that certain clinically relevant interactions between P-glycoprotein substrates and inhibitors and/or inducers depend on the route of administration. A number of clinically important drug interactions with rifampicin have been reported that are caused by the potent induction of intestinal CYP3A4.^[135,136] However, recent findings indicate that coadministration of rifampicin 600 mg/day for 10 days was associated with substantially reduced

digoxin plasma concentrations after oral administration, but to a lesser extent after intravenous administration.^[137] When duodenal biopsies were analysed before and after administration of rifampicin, the treatment was found to increase intestinal P-glycoprotein content 3.5-fold, which correlated with the extent of reduction of AUC after oral but not after intravenous administration of digoxin.^[137] These results suggest that intestinal P-glycoprotein plays a key role in the systemic availability of digoxin. Likewise, in the study conducted by Kurata et al.,^[11] coadministration of clarithromycin increased the digoxin AUC substantially after oral administration but to a lesser extent after intravenous administration. Interestingly, the AUC values of digoxin after intravenous administration were comparable among the three genotypic groups.

5. Conclusion

Although many factors, such as diet, race and disease state, may influence interindividual variability in the pharmacokinetic and pharmacodynamic outcomes of treatment with P-glycoprotein substrate drugs, the premise that genetic variations in the *MDR1* gene are one of the prime determinants of this variability is supported by a number of human studies. The clinical usefulness of genotyping would be expected to increase if it allowed a more accurate prediction of transport activity in humans. In order to achieve this, at least four points of research will be of importance.

Firstly, although the effects of *MDR1* gene variations on phenotypic indices (pharmacokinetics and pharmacodynamics) are controversial, most studies agree that P-glycoprotein expression correlates inversely with phenotype indices, e.g. less protein on the apical surface of intestinal enterocytes to pump substrates back into the intestinal lumen, resulting in increased bioavailability, and vice versa. Thus, additional studies of expression mechanisms (e.g. translation efficacy) with regard to *MDR1* gene variations are needed; expression levels can be influenced by structural differences in the genome, such as chromatin alterations and methylation.

Secondly, the identification of new functionally important mutations and/or haplotypes is needed to more accurately explain the variability in transport activity. The majority of *in vivo* data on the importance of *MDR1* polymorphisms in humans are from single-dose pharmacokinetic studies focused on a single polymorphism (e.g. G2677T/A or C3435T). Like many genes, the *MDR1* gene has multiple polymorphisms, some of which are in linkage disequilibrium. Thus, haplotypes or mutation patterns should be considered when clinical studies are conducted.

Thirdly, a 'candidate gene' pharmacogenomic approach,^[138] where polymorphisms in multiple genes known or suspected to contribute to drug responses and kinetics are considered, is also useful. Combined genotyping of the *MDR1* and *CYP2C9* genes, allowing a more accurate prediction of phenytoin (a substrate for both proteins) plasma concentrations, is one example.^[76]

Finally, rapid progress in the study of drug transporters in recent years has allowed us to identify the specific transporters involved in the disposition and distribution of certain drugs. For example, by use of recent technologies (e.g. site-directed mutagenesis and gene knockout in mice), digoxin was found to be a dual substrate for both P-glycoprotein and OATP-8, meaning that the contribution of both transporters with regard to genetic variation needs to be considered in order to describe more accurately the pharmacokinetics, and thus the clinical outcome, of digoxin treatment. As can be seen from this review, digoxin and fexofenadine may not be suitable substrates for *in vivo* pharmacogenetic testing. It is clear that the identification of specific probe drugs for P-glycoprotein is required.

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Note in Proof

Additional important works regarding *MDR1* polymorphisms have been published since acceptance of this review.^[139-141]

References

- Roberts RL, Joyce PR, Mulder RT, et al. A common P-glycoprotein polymorphism is associated with nortriptyline-induced postural hypotension in patients treated for major depression. *Pharmacogenetics* 2002; 2: 191-6
- Fellay J, Marzolini C, Meaden ER, et al. Response to antiretroviral treatment in HIV-1-infected individuals with allelic variants of the multidrug resistance transporter 1: a pharmacogenetic study. *Lancet* 2002; 359: 30-6
- Siegmund M, Brinkmann U, Schaffeler E, et al. Association of the P-glycoprotein transporter MDR1C3435T polymorphism with the susceptibility to renal epithelial tumors. *J Am Soc Nephrol* 2002; 13: 1847-54
- Illmer T, Schuler US, Thiede C, et al. MDR1 gene polymorphisms affect therapy outcome in acute myeloid leukemia patients. *Cancer Res* 2002; 62: 4955-62
- Zheng H, Webber S, Zeevi A, et al. The MDR1 polymorphisms at exon 21 and 26 predict steroid weaning in pediatric heart transplant patients. *Hum Immunol* 2002; 63: 765-70
- Yamauchi A, Ieiri I, Kataoka Y, et al. Neurotoxicity induced by tacrolimus after liver transplantation: relation to genetic polymorphisms of the ABCB1 (MDR1) gene. *Transplantation* 2002; 74: 571-8
- Goto M, Masuda S, Saito H, et al. C3435T polymorphism in the MDR1 gene affects the enterocyte expression level of CYP3A4 rather than Pgp in recipients of living-donor liver transplantation. *Pharmacogenetics* 2002; 12: 451-7
- Nakamura T, Sakaeda T, Horinouchi M, et al. Effect of the mutation (C3435T) at exon 26 of the MDR1 gene on expression level of MDR1 messenger ribonucleic acid in duodenal enterocytes of healthy Japanese subjects. *Clin Pharmacol Ther* 2002; 71: 297-303
- Hoffmeyer S, Burk O, von Richter O, et al. Functional polymorphisms of the human multidrug-resistance gene: multiple sequence variants and correlation of one allele with P-glycoprotein expression and activity *in vivo*. *Proc Natl Acad Sci U S A* 2000; 97: 3473-8
- Tanabe M, Ieiri I, Nagata N, et al. Expression of P-glycoprotein in human placenta: relation to genetic polymorphism of the multidrug resistance (MDR)-1 gene. *J Pharmacol Exp Ther* 2001; 297: 1137-43
- Kurata Y, Ieiri I, Kimura M, et al. Role of human MDR1 gene polymorphism in bioavailability and interaction of digoxin, a substrate of P-glycoprotein. *Clin Pharmacol Ther* 2002; 72: 209-29
- Siegmund W, Altmannsberger S, Pancitz A, et al. Effect of levothyroxine administration on intestinal P-glycoprotein expression: consequences for drug disposition. *Clin Pharmacol Ther* 2002; 72: 256-64
- Kim RB. MDR1 single nucleotide polymorphisms: multiplicity of haplotypes and functional consequences. *Pharmacogenetics* 2002; 12: 425-7
- Germann UA. P-glycoprotein: a mediator of multidrug resistance in tumor cells. *Eur J Cancer* 1996; 32A: 927-44
- Gottesman MM, Pastan I. Biochemistry of multidrug resistance mediated by the multidrug transporter. *Annu Rev Biochem* 1993; 62: 385-427
- Gottesman MM, Pastan I, Ambudkar SV. P-glycoprotein and multidrug resistance. *Curr Opin Genet Dev* 1996; 6: 610-7
- Goldstein LJ, Pastan I, Gottesman MM. Multidrug resistance in human cancer. *Crit Rev Oncol Hematol* 1992; 12: 243-53
- Cordon-Cardo C, O'Brien JP, Boccia J, et al. Expression of the multidrug resistance gene product (P-glycoprotein) in human normal and tumor tissues. *J Histochem Cytochem* 1990; 38: 1277-87
- Thiebaut F, Tsuruo T, Hamada H, et al. Cellular localization of the multidrug-resistance gene product P-glycoprotein in normal human tissues. *Proc Natl Acad Sci U S A* 1987; 84: 7735-8
- Borst P, Schinkel AH, Smith JJM, et al. Classical and novel forms of multidrug resistance and the physiological functions of P-glycoprotein in mammals. *Pharmacol Ther* 1993; 60: 289-99
- Fojo AT, Ueda K, Salmon DJ, et al. Expression of a multidrug-resistance gene in human tumors and tissues. *Proc Natl Acad Sci U S A* 1987; 84: 265-9
- Sugawara I, Kataoka I, Morishita Y, et al. Tissue distribution of P-glycoprotein encoded by a multidrug-resistance gene as revealed by a monoclonal antibody, MRK16. *Cancer Res* 1988; 48: 1926-9
- Chaudhary PM, Roninson IB. Expression and activity of P-glycoprotein, a multidrug efflux pump, in human hematopoietic stem cells. *Cell* 1991; 66: 85-94
- Klimecki WT, Futscher BW, Grogan TM, et al. P-glycoprotein expression and function in circulating blood cells from normal volunteers. *Blood* 1994; 83: 2451-8
- Kim RB, Fromm MF, Wandel C, et al. The drug transporter P-glycoprotein limits oral absorption and brain entry of HIV-1 protease inhibitors. *J Clin Invest* 1998; 101: 289-94
- Mayer U, Wagenaar E, Beijnen JH, et al. Substantial excretion of digoxin via the intestinal mucosa and prevention of long-term digoxin accumulation in the brain by mdrla P-glycoprotein. *Br J Pharmacol* 1996; 119: 1038-44
- Nakamura Y, Ikeda S, Furukawa T, et al. Function of P-glycoprotein expressed in placenta and mole. *Biochem Biophys Res Commun* 1997; 235: 849-53
- Schinkel AH, Wagenaar E, Mol CA, et al. P-glycoprotein in the blood-brain barrier of mice influences the brain penetration and pharmacological activity of many drugs. *J Clin Invest* 1996; 97: 2517-24
- Cordon-Cardo C, O'Brien JP, Casals D, et al. Multidrug-resistance gene (P-glycoprotein) is expressed by endothelial cells at blood-brain barrier sites. *Proc Natl Acad Sci U S A* 1989; 86: 695-8
- Schinkel AH, Mayer U, Wagenaar E, et al. Normal viability and altered pharmacokinetics in mice lacking mdrl-type (drug-transporting) P-glycoprotein. *Proc Natl Acad Sci U S A* 1997; 94: 4028-33
- Sparreboom A, van Asperen J, Mayer U, et al. Limited oral bioavailability and active epithelial excretion of paclitaxel (Taxol) caused by P-glycoprotein in the intestine. *Proc Natl Acad Sci U S A* 1997; 94: 2031-5
- Sugiyama Y, Kato Y, Chu X, et al. Multiplicity of biliary excretion mechanisms for the camptothecin derivative irinotecan (CPT-11), its metabolite SN-38, and its glucuronide: role of canalicular multispecific organic anion transporter and P-glycoprotein. *Cancer Chemother Pharmacol* 1998; 42 Suppl.: S44-9

33. Schuetz EG, Yasuda K, Arimori K, et al. Human MDR1 and mouse mdr1a P-glycoprotein alter the cellular retention and disposition of erythromycin, but not of retinoic acid or benzo(a)pyrene. *Arch Biochem Biophys* 1998; 350: 340-7
34. Ito T, Yano I, Tanaka K, et al. Transport of quinolone antibacterial drugs by human P-glycoprotein expressed in a kidney epithelial cell line, LLC-PK1. *J Pharmacol Exp Ther* 1997; 282: 955-60
35. Saeki T, Ueda K, Tanigawara Y, et al. Human P-glycoprotein transports cyclosporin A and FK506. *J Biol Chem* 1993; 268: 6077-80
36. de Lannoy IA, Silverman M. The MDR1 gene product P-glycoprotein, mediates the transport of the cardiac glycoside, digoxin. *Biochem Biophys Res Commun* 1992; 189: 551-7
37. Fromm MF, Kim RB, Stein CM, et al. Inhibition of P-glycoprotein-mediated drug transport: a unifying mechanism to explain the interaction between digoxin and quinidine. *Circulation* 1999; 99: 552-7
38. Saeki T, Ueda K, Tanigawara Y, et al. P-glycoprotein-mediated transcellular transport of MDR-reversing agents. *FEBS Lett* 1993; 324: 99-102
39. Pauli-Magnus C, von Richter O, Burk O, et al. Characterization of the major metabolites of verapamil as substrates and inhibitors of P-glycoprotein. *J Pharmacol Exp Ther* 2000; 293: 376-82
40. Alsenz J, Steffen H, Alex R. Active apical secretory efflux of the HIV protease inhibitors are substrates for the MDR1 multidrug transporter. *Pharm Res* 1998; 15: 423-8
41. Schwab M, Eichelbaum M, Fromm MF. Genetic polymorphisms of the human MDR1 drug transporter. *Annu Rev Pharmacol Toxicol* 2003; 43: 285-307
42. Fromm MF. The influence of MDR1 polymorphisms on P-glycoprotein expression and function in humans. *Adv Drug Deliv Rev* 2002; 54: 1295-310
43. Kim RB. Drugs as P-glycoprotein substrates, inhibitors, and inducers. *Drug Metab Rev* 2002; 34: 47-54
44. Wachter J, Wu CY, Benet LZ. Overlapping substrate specificities and tissue distribution of cytochrome P4503A4 and p-glycoprotein: implications for drug delivery and cancer chemotherapy. *Mol Carcinog* 1995; 13: 129-34
45. Kim RB, Wandel C, Leake B, et al. Interrelationship between substrates and inhibitors of human CYP3A and P-glycoprotein. *Pharm Res* 1999; 16: 408-14
46. Wachter VJ, Silverman JA, Zhang Y, et al. Role of P-glycoprotein and cytochrome P4503A in limiting oral absorption of peptides and peptidomimetic. *J Pharm Sci* 1998; 87: 1322-30
47. Sharom FJ. The P-glycoprotein efflux pump: how does it transport drugs? *J Membr Biol* 1997; 160: 161-75
48. Gottesman MM, Hrycyna CA, Schoenlein PV, et al. Genetic analysis of the multidrug transporter. *Annu Rev Genet* 1995; 29: 607-47
49. Mickley LA, Lee JS, Weng Z, et al. Genetic polymorphism in MDR-1: a tool for examining allelic expression in normal cells, unselected and drug-selected cell lines, and human tumors. *Blood* 1998; 91: 1749-56
50. Ito S, Ieiri I, Tanabe M, et al. Polymorphism of the ABC transporter genes, MDR1, MRP1 and MRP2/cMOAT, in healthy Japanese subjects. *Pharmacogenetics* 2001; 11: 175-84
51. Cascorbi I, Gerloff T, John A, et al. Frequency of single nucleotide polymorphisms in the P-glycoprotein drug transporter MDR1 gene in white subjects. *Clin Pharmacol Ther* 2001; 69: 169-74
52. Kim RB, Leake BF, Choo EF, et al. Identification of functionally variant MDR1 alleles among European Americans and African Americans. *Clin Pharmacol Ther* 2001; 70: 189-99
53. Siegmund W, Ludwig K, Giessmann T, et al. The effects of the human MDR1 genotype on the expression of duodenal P-glycoprotein and disposition of the probe drug talinolol. *Clin Pharmacol Ther* 2002; 72: 572-83
54. Tang K, Ngoi S-M, Gwee P-C, et al. Distinct haplotype profiles and strong linkage disequilibrium at the MDR1 multidrug transporter gene locus in three ethnic Asian populations. *Pharmacogenetics* 2002; 12: 437-50
55. Sakaeda T, Nakamura T, Horinouchi M, et al. MDR1 genotype-related pharmacokinetics of digoxin after single oral administration in healthy Japanese subjects. *Pharm Res* 2001; 18: 1400-4
56. Ameyaw M-M, Regateiro F, Li T, et al. MDR1 pharmacogenetics: frequency of the C3435T mutation in exon 26 is significantly influenced by ethnicity. *Pharmacogenetics* 2001; 11: 217-21
57. Chowbay B, Cumaraswamy S, Cheung YB, et al. Genetic polymorphisms in MDR1 and CYP3A4 genes in Asians and the influence of MDR1 haplotypes on cyclosporin disposition in heart transplant recipients. *Pharmacogenetics* 2003; 13: 89-95
58. Schaeffeler E, Eichelbaum M, Brinkmann U, et al. Frequency of C3435T polymorphism of MDR1 gene in African people. *Lancet* 2001; 358: 383-4
59. Furuno T, Landi MT, Ceroni M, et al. Expression polymorphism of the blood-brain barrier component P-glycoprotein (MDR1) in relation to Parkinson's disease. *Pharmacogenetics* 2002; 12: 529-34
60. von Ahnen N, Richter M, Grupp C, et al. No influence of the MDR-1 C3435T polymorphism or a CYP3A4 promoter polymorphism (CYP3A4-V allele) on dose-adjusted cyclosporin A through concentrations or rejection incidence in stable renal transplant recipients. *Clin Chem* 2001; 47: 1048-52
61. Honda T, Dan Y, Koyabu N, et al. Polymorphism of MDR1 gene in healthy Japanese subjects: a novel SNP with an amino acid substitution (Glu108Lys). *Drug Metab Pharmacokinet* 2002; 17: 479-81
62. Lindholm A, Welsh M, Alton C, et al. Demographic factors influencing cyclosporine pharmacokinetic parameters in patients with uremia: racial differences in bioavailability. *Clin Pharmacol Ther* 1992; 52: 359-71
63. Lown KS, Mayo RR, Leichtman AB, et al. Role of intestinal P-glycoprotein (mdr1) in interpatient variation in the oral bioavailability of cyclosporine. *Clin Pharmacol Ther* 1997; 62: 248-60
64. Frassetto L, Mancinelli L, Christians U, et al. Fluconazole-induced changes in tacrolimus oral bioavailability in three ethnic groups [abstract]. *Millennial World Congress of Pharmaceutical Sciences*; 2000 Apr 16-20; San Francisco
65. Mancinelli LM, Frassetto LM, Floren LC, et al. The pharmacokinetics and metabolic disposition of tacrolimus: a comparison across ethnic groups. *Clin Pharmacol Ther* 2001; 69: 24-31
66. Fitzsimmons WE, Bekersky I, Dressler D, et al. Demographic considerations in tacrolimus pharmacokinetics. *Transplant Proc* 1998; 30: 1359-64
67. Elmore JG, Moceri VM, Carter D, et al. Breast carcinoma tumor characteristics in black and white women. *Cancer* 1998; 83: 2509-15
68. Panwala CM, Jones JC, Viney JL. A novel model if inflammatory bowel disease: mice deficient for the multiple drug resis-

- tance gene, *mdr1a*, spontaneously develop colitis. *J Immunol* 1998; 161: 5733-44
69. Maggio-Price L, Shows D, Waggle K, et al. *Helicobacter bilis* infection accelerates and *H. hepaticus* infection delays the development of colitis in multiple drug resistance-deficient (*mdr1a*^{-/-}) mice. *Am J Pathol* 2002; 160: 739-51
 70. Schwab M, Schaeffeler E, Marx C, et al. Association between the C3435T MDR1 gene polymorphism and susceptibility for ulcerative colitis. *Gastroenterology* 2003; 124: 26-33
 71. Rund D, Azar I, Shperling O. A mutation in the promoter of the multidrug resistance gene (MDR1) in human hematological malignancies may contribute to the pathogenesis of resistant disease. In: Kaspers GJL, Pieters RP, Veerman AJP, editors. *Drug resistance in leukemia and lymphoma III*. New York: Plenum Publishers, 1999: 71-5
 72. Stein U, Walther W, Wunderlich V. Point mutations in the *mdr1* promoter of human osteosarcomas are associated with *in vitro* responsiveness to multidrug resistance relevant drugs. *Eur J Cancer* 1994; 30A: 1541-5
 73. Cornwell MM, Smith DE. SP1 activates the MDR1 promoter through one of two distinct G-rich regions that modulate promoter activity. *J Biol Chem* 1993; 268: 19505-11
 74. Cohen D, Yu L, Rzepka R, et al. Identification of two nuclear protein binding sites and their role in the regulation of the murine multidrug resistance *mdr1a* promoter. *DNA Cell Biol* 1994; 13: 641-9
 75. van Groenigen M, Valentijn L, Baas F. Identification of a functional initiator sequence in the human MDR1 promoter. *Biochim Biophys Acta* 1993; 1172: 138-46
 76. Kerb R, Aynacioglu AS, Brockmoller J, et al. The predictive value of MDR1, CYP2C9, and CYP2C19 polymorphisms for phenytoin plasma levels. *Pharmacogenomics J* 2001; 1: 204-10
 77. Min DI, Ellingrod VL. C3435T mutation in exon 26 of the human MDR1 gene and cyclosporine pharmacokinetics in healthy subjects. *Ther Drug Monit* 2002; 24: 400-4
 78. John A, Kopke K, Gerloff T, et al. Modulation of steady-state kinetics of digoxin by haplotypes of the P-glycoprotein MDR1 gene. *Clin Pharmacol Ther* 2002; 72: 584-94
 79. Drescher S, Schaeffeler E, Hitzl M, et al. MDR1 gene polymorphisms and disposition of the P-glycoprotein substrate fexofenadine. *Br J Clin Pharmacol* 2002; 53: 526-34
 80. Goh BC, Lee SC, Wang LZ, et al. Explaining interindividual variability of docetaxel pharmacokinetics and pharmacodynamics in Asians through phenotyping and genotyping strategies. *J Clin Oncol* 2002; 20: 3683-90
 81. Potocnik U, Ravnik-Glavac M, Golouh R, et al. Naturally occurring mutations and functional polymorphisms in multidrug resistance 1 gene: correlation with microsatellite instability and lymphoid infiltration in colorectal cancers. *J Med Genet* 2002; 39: 340-6
 82. Hitzl M, Drescher S, van der Kuip H, et al. The C3435T mutation in the human MDR1 gene is associated with altered efflux of the P-glycoprotein substrate rhodamine 123 from CD34⁺ natural killer cells. *Pharmacogenetics* 2001; 11: 293-8
 83. Calado RT, Falcao RP, Garcia AB, et al. Influence of functional MDR1 gene polymorphisms on P-glycoprotein activity in CD34⁺ hematopoietic stem cells. *Haematologica* 2002; 87: 564-8
 84. Meissner K, Sperker B, Karsten C, et al. Expression and localization of P-glycoprotein in human heart: effects of cardiomyopathy. *J Histochem Cytochem* 2002; 50: 1351-6
 85. Drach J, Gsur A, Hamilton G, et al. Involvement of P-glycoprotein in the transmembrane transport of interleukin-2 (IL-2), IL-4, and interferon-gamma in normal human T lymphocytes. *Blood* 1996; 88: 1747-54
 86. Klimecki WT, Taylor CW, Dalton WS. Inhibition of cell-mediated cytotoxicity and P-glycoprotein function in natural killer cells by verapamil isomers and cyclosporine A analogs. *J Clin Immunol* 1995; 15: 152-8
 87. Cvetkovic M, Leake B, Fromm MF, et al. OATP and P-glycoprotein transporters mediate the cellular uptake and excretion of fexofenadine. *Drug Metab Dispos* 1999; 27: 866-71
 88. Tirona RG, Kin RB. Pharmacogenomics of organic anion-transporting polypeptides (OATP). *Adv Drug Deliv Rev* 2002; 54: 1343-52
 89. Kimchi-Sarfaty C, Griber JJ, Gottesman MM. Functional characterization of coding polymorphisms in the human MDR1 gene using a vaccinia virus expression system. *Mol Pharmacol* 2002; 62: 1-6
 90. Kullak-Ublick GA, Ismail MG, Stieger B, et al. Organic anion-transporting polypeptide B (OATP-B) and its functional comparison with three other OATPs of human liver. *Gastroenterology* 2001; 120: 525-33
 91. Suzuki A, Tirona RG, Leake B, et al. Polymorphisms in the digoxin uptake transporter OATP-8, among Japanese, African-, and European-American subjects [abstract]. *Clin Pharmacol Ther* 2002; 71: P104
 92. Tirona RG, Leake BF, Merino G, et al. Polymorphisms in OATP-C: identification of multiple allelic variants associated with altered transport activity among European- and African-Americans. *J Biol Chem* 2001; 276 (38): 35669-75
 93. Nishizato Y, Ieiri I, Suzuki H, et al. Polymorphisms of OATP-C (SLC21A6) and OAT3 (SLC22A8) genes: consequences for pravastatin pharmacokinetics. *Clin Pharmacol Ther* 2003; 73: 554-65
 94. Bailey DG, Malcolm J, Arnold O, et al. Grapefruit juice-drug interactions. *Br J Clin Pharmacol* 1998; 46: 101-10
 95. Dresser GK, Bailey DG, Leake BF, et al. Fruit juices inhibit organic anion transporting polypeptide-mediated drug uptake to decrease the oral availability of fexofenadine. *Clin Pharmacol Ther* 2002; 71: 11-20
 96. Kobayashi D, Nozawa T, Imai K, et al. Involvement of human organic anion transporting polypeptide OATP-B (SLC21A9) in pH-dependent transport across intestinal apical membrane. *J Pharmacol Exp Ther* 2003; 306: 703-8
 97. Tamai I, Nezu J, Uchino H, et al. Molecular identification and characterization of novel members of the human organic anion transporter (OATP) family. *Biochem Biophys Res Commun* 2000; 273: 251-60
 98. Takanaga H, Ohnishi A, Matsuo H, et al. Inhibition of vinblastine efflux mediated by P-glycoprotein by grapefruit juice component in Caco-2 cells. *Biol Pharm Bull* 1998; 21: 1062-6
 99. Becquemont L, Verstuyft C, Kerb R, et al. Effect of grapefruit juice on digoxin pharmacokinetics in human. *Clin Pharmacol Ther* 2001; 70: 311-6
 100. Judson R, Stephens JC, Windemuth A. The predicted power of haplotypes in clinical response. *Pharmacogenomics* 2000; 1: 15-26
 101. Stephens JC, Schneider JA, Tanguay DA, et al. Haplotype variation and linkage disequilibrium in 313 human genes. *Science* 2001; 293: 489-93
 102. Nakayama M, Wada M, Harada T, et al. Hypomethylation status of CpG sites at the promoter region and overexpression

- of the human MDR1 gene in acute myeloid leukemias. *Blood* 1998; 92: 4296-307
103. Mickley LA, Lee JS, Weng Z, et al. Gene rearrangement: a novel mechanism for MDR-1 gene activation. *J Clin Invest* 1997; 99: 1947-57
 104. Boyes J, Bird A. DNA methylation inhibits transcription indirectly via a methyl-CpG binding protein. *Cell* 1991; 64: 1123-34
 105. Laird PW, Jaenisch R. DNA methylation and cancer. *Hum Mol Genet* 1994; 3: 1487-95
 106. Garcia-Manero G, Bueso-Ramos C, Daniel J, et al. DNA methylation patterns at relapse in adult acute lymphocytic leukemia. *Clin Cancer Res* 2002; 8: 1897-903
 107. Tada Y, Wada M, Kuroiwa K, et al. MDR1 gene over-expression and altered degree of methylation at the promoter region in bladder cancer during chemotherapeutic treatment. *Clin Cancer Res* 2000; 6: 4618-27
 108. Kusaba H, Nakayama M, Harada T, et al. Association of 5' CpG demethylation and altered chromatin structure in the promoter region with transcriptional activation of the multidrug resistance 1 gene in human cancer cells. *Eur J Biochem* 1999; 262: 924-32
 109. Kantharidis P, El-Osta A, deSilva M, et al. Altered methylation of the human MDR1 promoter is associated with acquired multidrug resistance. *Clin Cancer Res* 1997; 3: 2025-32
 110. Lucia MB, Cauda R, Landay AL, et al. Transmembrane P-glycoprotein (P-gp/P-170) in HIV infection: analysis of lymphocyte surface expression and drug-unrelated function. *AIDS Res Hum Retroviruses* 1995; 11: 893-901
 111. Smit JW, Schinkel AH, Weert B, et al. Hepatobiliary and intestinal clearance of amphiphilic cationic drugs in mice in which both *mdr1a* and *mdr1b* genes have been disrupted. *Br J Pharmacol* 1998; 124: 416-24
 112. Schuetz EG, Umbenhauer DR, Yasuda K, et al. Altered expression of hepatic cytochromes P-450 in mice deficient in one or more *mdr1* genes. *Mol Pharmacol* 2000; 57: 188-97
 113. Srinivas RV, Middlemas D, Flynn P, et al. Human immunodeficiency virus protease inhibitors serve as substrates for multidrug transporter proteins MDR1 and MRP1 but retain antiviral efficacy in cell lines expressing these transporters. *Antimicrob Agents Chemother* 1998; 42: 3157-62
 114. Gutmann H, Fricker G, Drewe J, et al. Interactions of HIV protease inhibitors with ATP-dependent drug export proteins. *Mol Pharmacol* 1999; 56: 383-9
 115. Carrillo JA, Ramos SI, Agundez JA, et al. Analysis of midazolam and metabolites in plasma by high-performance liquid chromatography: probe of CYP3A. *Ther Drug Monit* 1998; 20: 319-24
 116. Hashida T, Masuda S, Uemoto S, et al. Pharmacokinetic and prognostic significance of intestinal MDR1 expression in recipients of living-donor liver transplantation. *Clin Pharmacol Ther* 2001; 69: 308-16
 117. Schaich M, Pitter M, Illmer T, et al. Mutations in ras proto-oncogenes are associated with lower *mdr1* gene expression in adult acute myeloid leukaemia. *Br J Haematol* 2001; 112: 300-7
 118. Relling MV. Are the major effects of P-glycoprotein modulators due to altered pharmacokinetics of anticancer drugs? *Ther Drug Monit* 1996; 18: 350-6
 119. Bart J, Groen HJ, Hendrikse NH, et al. The blood-brain barrier and oncology: new insights into function and modulation. *Cancer Treat Rev* 2000; 26: 449-62
 120. Baekelandt MM, Holm R, Nesland JM, et al. P-glycoprotein expression is a marker for chemotherapy resistance and prognosis in advanced ovarian cancer. *Anticancer Res* 2000; 20: 1061-7
 121. Van der Heuvel-Eibrink MM, Sonneveld P, Pieters R. The prognostic significance of membrane transport-associated multidrug resistance (MDR) proteins in leukemia. *Int J Clin Pharmacol Ther* 2000; 38: 94-110
 122. Lazarowski A, Sevlever G, Taratuto A, et al. Tuberous sclerosis associated with MDR1 gene expression and drug-resistant epilepsy. *Pediatr Neurol* 1999; 21: 731-4
 123. Dhooze C, De Moerloose B, Laurey G, et al. P-glycoprotein is an independent prognostic factor predicting relapse in childhood acute lymphoblastic leukaemia: results of a 6-year prospective study. *Br J Haematol* 1999; 105: 676-83
 124. Chaudhary PM, Mechetner EB, Robinson IB. Expression and activity of the multidrug resistance P-glycoprotein in human peripheral blood lymphocytes. *Blood* 1992; 80: 2735-9
 125. Coon JS, Wang Y, Bines SD, et al. Multidrug resistance activity in human lymphocytes. *Hum Immunol* 1991; 32: 134-40
 126. Kernnitz J, Uysal A, Haverich A, et al. Multidrug resistance in heart transplant patients: a preliminary communication on possible mechanisms of therapy-resistant rejection. *J Heart Lung Transplant* 1991; 10: 201-10
 127. Zanker B, Barth C, Stachowski J, et al. Multidrug resistance gene MDR1 expression: a gene transfection *in vitro* model and clinical analysis in cyclosporine-treated patients rejecting their grafts. *Transplant Proc* 1997; 29: 1507-8
 128. Midoneck SR, Etingin OR. Clarithromycin-related toxic effects of digoxin [letter]. *N Engl J Med* 1995; 333: 1505
 129. Brown BA, Wallace Jr RJ, Griffith DE, et al. Clarithromycin-associated digoxin toxicity in the elderly. *Clin Infect Dis* 1997; 24: 92-3
 130. Nawarskas JJ, McCarthy DM, Spinler SA. Digoxin toxicity secondary to clarithromycin therapy. *Ann Pharmacother* 1977; 31: 864-6
 131. Wakasugi H, Yano I, Ito T, et al. Effect of clarithromycin on renal excretion of digoxin: interaction with P-glycoprotein. *Clin Pharmacol Ther* 1998; 64: 123-8
 132. Yu KS, Yim DS, Cho JY, et al. Effect of omeprazole on the pharmacokinetics of moclobemide according to the genetic polymorphism of CYP2C19. *Clin Pharmacol Ther* 2001; 69: 266-73
 133. Andersson T, Cederberg C, Edvardsson G, et al. Effect of omeprazole treatment on diazepam plasma levels in slow versus normal rapid metabolizers of omeprazole. *Clin Pharmacol Ther* 1990; 47: 79-85
 134. Funck-Brentano C, Becquemont L, Leneveu A, et al. Inhibition by omeprazole of proguanil metabolism: mechanism of the interaction *in vitro* and prediction of *in vivo* results from the *in vitro* experiments. *J Pharmacol Exp Ther* 1997; 280: 730-8
 135. Fromm MF, Busse D, Kroemer HK, et al. Differential induction of prehepatic and hepatic metabolism of verapamil by rifampin. *Hepatology* 1996; 24: 796-801
 136. Krishna DR, Klotz U. Extrahepatic metabolism of drugs in humans. *Clin Pharmacokinet* 1994; 26: 144-60
 137. Greiner B, Eichelbaum M, Fritz P, et al. The role of intestinal P-glycoprotein in the interaction of digoxin and rifampin. *J Clin Invest* 1999; 104: 147-53
 138. Evans WE, Johnson JA. Pharmacogenomics: the inherited basis for interindividual differences in drug response. *Annu Rev Genomics Hum Genet* 2001; 2: 9-39

139. Sai K, Kaniwa N, Itoda M, et al. Haplotype analysis of ABCB1/MDR1 blocks in a Japanese population reveals genotype-dependent renal clearance of irinotecan. *Pharmacogenetics* 2003; 13: 741-57
140. Kroetz DL, Pauli-Magnus C, Hodges LM, et al. Sequence diversity and haplotype structure in the human *ANCN1* (MDR1, multidrug resistance transporter) gene. *Pharmacogenetics* 2003; 13: 481-94
141. Skarke C, Jarrar M, Schmidt H, et al. Effects of ABCB1 (multidrug resistance transporter) gene mutations on disposi-

tion and central nervous effects of loperamide in healthy volunteers. *Pharmacogenetics* 2003; 13: 651-60

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